American Journal of Hematology 57:331–337 (1998)

T-Cell Lymphoma Associated With Natural Killer-Like T-Cell Reaction

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We report a case of T-cell lymphoma showing in the peripheral blood (PB) exclusively T-lymphocytes with suppresser T-cell preponderance and a high percentage of natural killer (NK) marker positive cells by flow cytometry. A T-cell receptor (TCR) gene analysis of the PB leukocytes demonstrated rearrangements of TCRα, TCRβ, and TCRγ genes. Therefore, the phenotype and genotype appeared to be consistent with an NK-like T-cell leukemia/lymphoma. However, when the PB lymphocytes were separated by size, it was found that 80% of NK marker positive cells were in the smaller cell population, while the neoplastic cells were in the large cell gate. A diagnosis of T-cell lymphoma with reactive NK-like T-cells was finally confirmed by demonstrating the presence of both large atypical lymphoid cells and large granular lymphocytes (LGL) on PB smears. Although immunoperoxidase stain of bone marrow and colon showed positive T-cell markers in the tumor cell population, cytoplasmic granules could not be identified in tissue sections and, thus, a distinction between T-cell lymphoma and NK-like T-cell lymphoma could not be made by light microscopy until NK markers were studied. CD57 was demonstrated immunohistochemically in small lymphocytes but not in the large tumor cells in the colon. Electron microscopy, however, demonstrated LGL reaction to the lymphoma cells in the colonic biopsy. NK-like T-cell lymphoma usually carries a poorer prognosis than peripheral T-cell lymphoma, thus the distinction of these neoplasms is important. This study emphasizes that T-cell lymphoma may cause an LGL reaction or proliferation. If the lymphoma cells were of the same size as LGL, flow cytometric studies may have misled the diagnosis to NK-like T-cell-lymphoma. Am. J. Hematol. 57:331–337, 1998.

Key words: T-cell lymphoma; NK-like T-cell; flow cytometry; gene rearrangement; immunohistochemistry

INTRODUCTION

Large granular lymphocytosis can be due to the proliferation of natural killer (NK) cells or NK-like T-cells. Although both populations carry NK markers, the former group shows no surface CD3 antigen and T-cell receptor (TCR) gene rearrangement, thus distinguishing it from the latter group, which is positive for CD3 and TCR gene rearrangement [1]. On this basis, large granular lymphocyte (LGL) proliferative disorders are divided into NK-LGL leukemia, T-LGL leukemia, and polyclonal LGL lymphocytosis [2]. Clinically, LGL disorders may present as chronic lymphocytic leukemia, acute lymphocytic leukemia, and lymphoma, regardless of whether the immunophenotype is of NK cell or NK-like T-cell [3]. The lymphoma type is frequently associated with a leukemic

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Received for publication 30 April 1997; Accepted 12 November 1997

TABLE I. Results of Immunohistochemical Studies on Tumor Cells*

Antibodies used (manufacturer)	Cluster designation	Cell identified	Bone marrow	Colon
CD3 (Dako, Carpinteria, CA)	CD3	T-cell	Positive	Not done
L26 (Dako)	CD20	B-cell	Not done	Negative
Leu 22 (Becton Dickinson, San Jose, CA)	CD43	T-cell	Positive	Positive
LCA (Dako)	CD45	Pan-leukocyte	Positive	Positive
UCHL1 (Dako)	CD45RO	T-cell	Not done	Positive
Leu 7 (Becton Dickinson)	CD57	NK, T-cell subset	Not done	Negative
LN-2 (ICN, Irvine, CA)	CD74	B-cell	Negative	Not done
OPD-4 (Dako)	CD4	Helper/inducer T-cell	Positive	Positive
DBA-44 (Dako)	—	Hairy cell leukemia, some T-cell lymphoma	Negative	Not done
S-100 (Dako)	—	Melanoma, some carcinomas	Negative	Not done

*The reactions of the reactive population are not included.

phase and an aggressive clinical course [4,5]. This type is considered as a distinct clinicopathologic entity, designated aggressive natural killer cell leukemia/lymphoma by Imamura et al. [4]. A diagnostic dilemma is that peripheral LGL proliferation can be a response to various conditions [6–8] and the demonstration of a high percentage of NK-positive cells in the peripheral blood of a lymphoma case may be misinterpreted as NK or NK-like T-cell lymphoma. We report an unusual case with 80% of NK-like T-cells in the peripheral blood and in the colonic biopsy as a reaction to a T-cell lymphoma, a condition that has not yet been documented.

CASE REPORT

A 67-year-old woman was found to have pancytopenia and decreased hematocrit by her family physician and was admitted to the hospital for a complete work-up. A bone marrow biopsy showed an atypical interstitial lymphoid infiltrate. A computed tomographic scan revealed lymphadenopathy in the abdomen. A barium enema demonstrated a questionable mass in the colon and a stool guaiac test was positive. Colonoscopy showed 2–4-mm polyps in the sigmoid colon and multiple 3-mm excrescences in the cecum. A biopsy of the polyps confirmed the diagnosis of lymphoma. During the series of examinations, her leukocyte count increased to 16.9×10^9/L with 78% lymphocytes. The patient was then discharged and treated with two cycles of cytoxan/vincristine/prednisone on an outpatient basis. With the exception of mild anorexia and fatigue, she was doing well until she developed fever, chills, and rigors. She was then readmitted for the second time.

Physical examination on the second admission revealed a shivering female with a temperature of 99.4°F. There was no peripheral lymphadenopathy or hepatosplenomegaly. Her hematologic work-up showed a total leukocyte count of 1.3×10^9/L, with 20% neutrophils and 73% lymphocytes. Her platelet count was 22×10^9/L, hemoglobin 8.2% g, and hematocrit 23%. Her blood chemistries were unremarkable except for a lactate dehydrogenase of 772 U/L, blood urea nitrogen of 56 mg/dl, and creatinine of 2.4 mg/dl. Her blood culture grew *Staphylococcus.*

The patient was placed on broad spectrum antibiotics, and treated with Neupogen. However, she became progressively lethargic and weak with progressive deterioration of her overall status. She eventually expired on the 21st hospital day.

MATERIALS AND METHODS

Electron Microscopy

Electron microscopic studies were done on a rehydrated, paraffin-retrieved specimen of the colon, using standard methodologies.

Immunohistochemistry

Immunohistochemical studies using a three-step avidin-biotin-peroxidase technique [9] were performed on paraffin sections of the bone marrow and colonic biopsies. A panel of monoclonal antibodies was used to characterize the tumor cells (Table I).

Flow Cytometry

Immunophenotyping was performed with the FACScan flow cytometer (Becton-Dickinson, San Jose, CA), using a panel of monoclonal antibodies as listed in Table II. Mononuclear cells were isolated by the standard Ficoll-Hypaque technique from the bone marrow aspirate and peripheral blood specimen.

Cytochemistry

The terminal deoxynucleotidyl transferase (TdT) stain was performed on the bone marrow aspirate by an im-

TABLE II. Immunophenotyping by Flow Cytometry (% Markers)*

Antibodies used	Cluster designation	Cell identified	Bone marrow		Blood	
			Small	Large	Small	Large
Anti-κ	—	B-cell	10	0	3	3
Anti-λ	—	B-cell	13	0	1	1
Leu 4	CD3	T-cell	76	6	94	96
Leu 3	CD4	Helper/inducer T-cell	ND	ND	13	6
Leu 1	CD5	T-cell, B-cell	75	4	93	95
Leu 9	CD7	T-cell	76	2	99	99
Leu 2	CD8	Cytotoxic/suppressor T-cell	ND	ND	33	35
Leu M7	CD13	Myeloid cell	82	0	1	1
Leu M4	CD14	Myeloid cell	80	1	0	0
Leu 11	CD16	Fc receptor of IgG	[a]	[a]	2	2
Leu 12	CD19	B-cell	4	3	1	1
Leu 16	CD20	B-cell	21	4	ND	ND
Leu M9	CD33	Myeloid cell	0	83	0	0
HPCA-1	CD34	Human progenitor cell	0	1	0	0
Leu 19	CD56	NK, T-cell subset	[a]	[a]	6	5
Leu 7	CD57	NK, T-cell subset	ND	ND	80	17
HLA-DR	—	B, activated T, myeloid cells	13	89	3	3
TCRαβ	—	TCRαβ antigen	ND	ND	97	97
TCRγδ	—	TCRγδ antigern	ND	ND	0	0

*Small = small cell population; Large = large cell population; ND = not done, NK = natural killer cells. All monoclonal antibodies were from Becton Dickinson (San Jose, CA).

[a]NK markers were tested as two panels: CD3- CD16+ CD56+: Small 17%, Large 25%; CD3+ CD16+ CD56+: Small 7%, Large 2%.

munofluorescence technique [10]. Myeloperoxidase stain [10] was also performed on the bone marrow aspirate.

Immunogenotyping

High molecular weight DNA was extracted from the peripheral blood specimen, digested with Bam HI, Hind III, Eco RI, and PstI, electrophoresed on 0.8% agarose gel, and then transferred to nitrocellulose membrane for hybridization [11]. DNA probes, including the constant region probes of T-cell receptor β and γ chain genes, were obtained from Oncore (Bethesda, MD). Probes for TCRα chain gene were provided by Dr. Tak W. Mak of Ontario Cancer Institute, Toronto, Canada. The J region probe for heavy chain gene and constant region probes for kappa and lambda light chain gene used in this study were described previously [12].

RESULTS

Morphology

Bone marrow. The marrow was hypercellular with erythroid hyperplasia and an interstitial infiltrate of small and large atypical mononuclear cells with abundant cytoplasm (Fig. 1). There was a mild diffuse increase in reticulin. The bone marrow touch preparation showed occasional large lymphoid-appearing cells with oval nuclei, coarse chromatin, occasional nucleoli, and abundant blue-gray cytoplasm with cytoplasmic vacuoles, admixed with hematopoietic elements.

Colonic biopsies. The colonic mucosa was focally infiltrated by atypical intermediate-sized and large lymphoid cells (Fig. 2). The large cells had oval nuclei with prominent nucleoli, and were associated with intermediate-sized cells with indented nuclei. Numerous small well-differentiated lymphocytes were admixed with these cells. The infiltrate was similar to that seen in the prior bone marrow biopsy.

Electron microscopic examination of the colonic tissue shows atypical large pleomorphic lymphoid cells intermingled with smaller lymphocytes with or without cytoplasmic granules. The latter population was consistent with large granular lymphocytes.

Peripheral blood smear. Large atypical lymphoid cells with or without prominent nucleoli and large granular lymphocytes were demonstrated in Wright/Giemsa stained preparations (Fig. 3).

Immunohistochemistry

Immunoperoxidase study of the bone marrow biopsy showed the mononuclear infiltrate to be positive for CD45, CD3, OPD-4, and CD43, but negative for CD74, DBA-44, myeloperoxidase, and S-100 (Table I). The colonic biopsy was positive for CD45 (Fig. 2), CD45RO, and CD43, but was negative for CD20 in the atypical lymphoid cells. The reactive NK-like T-cells were identified retrospectively by an anti-CD57 monoclonal antibody (Leu 7).

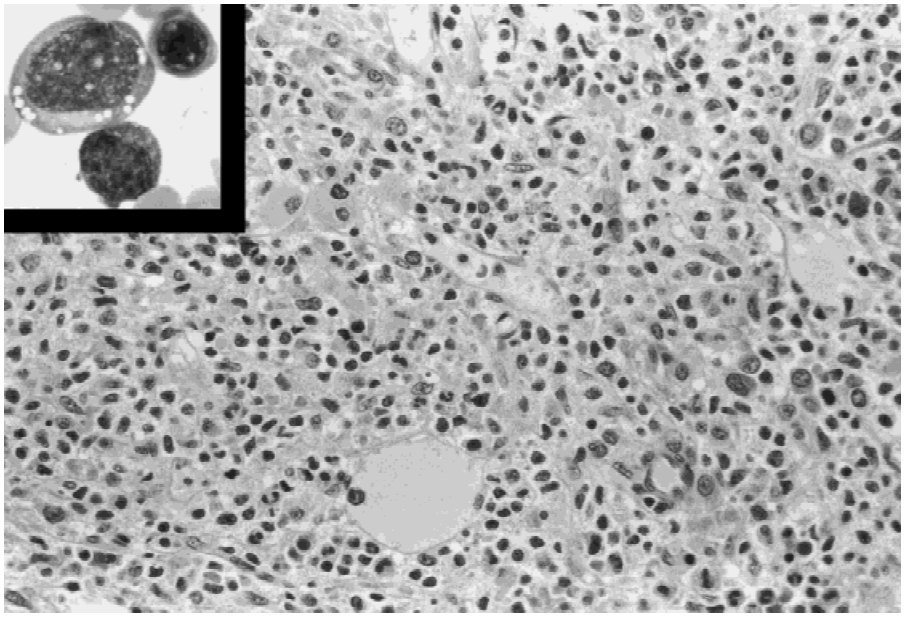

Fig. 1. Bone marrow biopsy showing interstitial infiltration of medium-sized lymphoid cells with irregular nuclei and moderate cytoplasm, intermingled with some residual hematopoietic cells, ×400 (inset). Touch preparation showing a large tumor cell with nucleoli and abundant vacuolated cytoplasm; a myelocyte and an erythroid precursor are present. Wright-Giemsa stain, ×1,000.

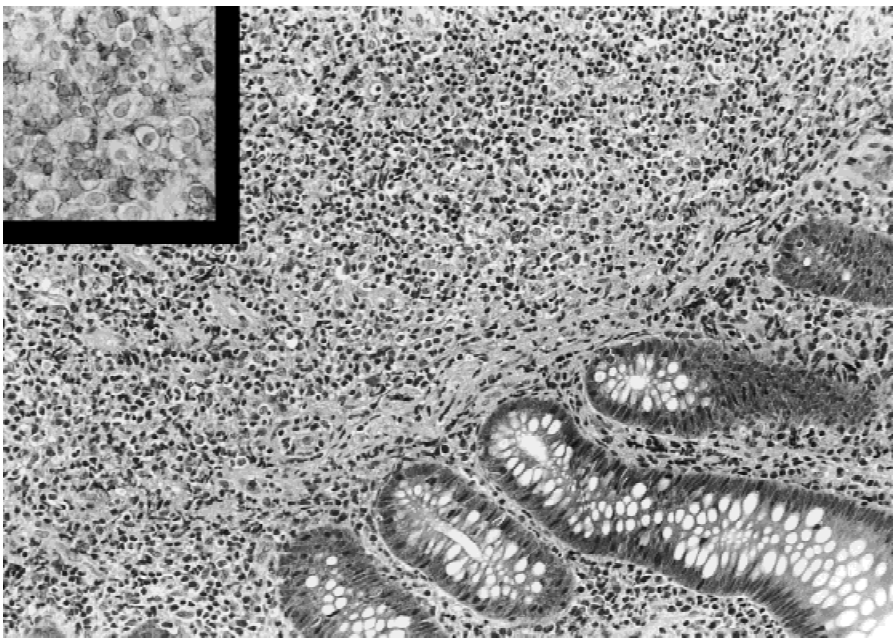

Fig. 2. Colonic biopsy showing focal infiltration by intermediate-sized and large lymphoid cells in the mucosa, ×200 (inset). Immunoperoxidase stain of colonic mucosa with CD45 monoclonal antibody showing positive surface membrane staining of lymphoid cells. ×400.

Flow Cytometry

The results of the flow cytometric analysis of the bone marrow specimen are listed in Table II. The large cell population showed mainly myeloid markers (CD13 82%, CD14 80%, CD33 83%). B- and T-cell marker positive cells were negligible but HLA-DR was 89%. A mixed antibody panel specific for CD3−/CD16+,CD56+ was reactive to 25% of the larger cells (representing NK cells) and the panel specific for CD3+/CD16+,CD56+ was re-

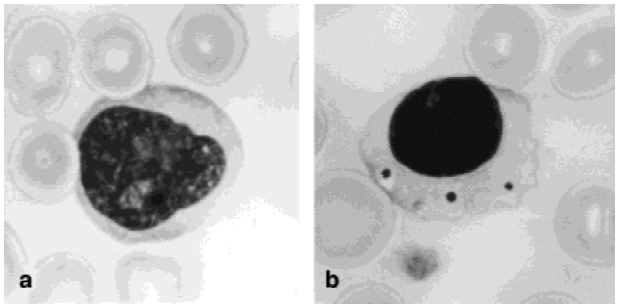

Fig. 3. Peripheral blood smear showing (a) a large tumor cells with a prominent nucleolus, and (b) a large granular lymphocyte with 3 prominent cytoplasmic granules. ×1,000.

active to 2% of larger cells (NK-line T-cells). The smaller cell population showed predominantly T-cell markers with a small percentage of B-cell markers and no myeloid markers. The NK cells and NK-like T-cells were 17 and 7%, respectively.

The peripheral blood specimen showed an almost exclusively T-cell population (93–99% positive for CD3, CD5, and CD7) in both larger and smaller cell categories. All T-cells carried the TCR$\alpha\beta$ dimer. The CD4/CD8 ratios in larger cell and smaller cell populations were 6:35 and 13:35, respectively. The major difference between these two populations was with the CD57 marker, which was present on 7% of the larger cell and 80% of the smaller cell population. Essentially, no leukocytes were stained for B-cell and myeloid markers.

Cytochemistry

Both TdT and myeloperoxidase stains were negative for the atypical mononuclear cells in the bone marrow aspirate.

Immunogenotyping

Immunogenotyping performed on the peripheral blood showed rearrangements of the TCRα,β, and γ genes (Fig. 4), and germline configuration for the immunoglobulin genes.

DISCUSSION

The most frequently encountered LGL disorder is the chronic leukemia type, which usually consists of an NK-like T-cell population [2,13]. This group of cases has a major phenotype of CD3+, CD4−, CD8+, CD16, and/or CD57+, CD56−, TCR protein + and consistent TCR gene rearrangement [13]. Therefore, the phenotype and genotype of the peripheral leukocytes in our case fit the criteria of a LGL disorder. When the LGL disorder is in the lymphoma category, the prognosis becomes ominous [3–5]. Our patient, indeed, had a rapidly progressive clinical course mimicking an aggressive natural killer cell leukemia/lymphoma as described by Imamura et al. [4]. Fortunately, the lymphoma cells were larger than the LGLs, so that flow cytometric gating readily separated the neoplastic from the reactive components in our case. After gating, it became apparent that the NK marker (CD57) was mainly expressed by the reactive NK-like T-cells.

The major disadvantage of flow cytometry is the lack of direct morphologic correlation with immunophenotyping. Should the tumor cells have the same size as the LGLs, their phenotypes could not have been distinguished. Therefore, we want to emphasize the importance of routine microscopic examination of the cell suspension that is subjected to flow cytometric analysis. This is particularly true if the source is from a tissue biopsy, because cytoplasmic granules cannot be identified in histologic sections. One should be cautioned, however, that the NK-like T-cells can be agranular [2,5,14], so that morphologic identification of NK-like T-cells may become problematic. Rearrangement of TCR genes in this case did not help, because it could not pinpoint whether or not the monoclonal population was identical to that of large granular lymphocytes.

The T-cell nature of this lymphoma was first suggested by immunoperoxidase stain of the atypical lymphoid cells in the bone marrow, but the small number of large lymphoma cells in the bone marrow was not picked up by flow cytometry. A diagnosis of T-cell lymphoma was eventually confirmed by the demonstration of the T-cell phenotype of the lymphoma cells infiltrating the colonic mucosa. Large granular lymphocytes cannot be identified morphologically in histologic sections; thus, their identity frequently depends on electron microscopy or tissue imprints [5]. In this case, tissue imprints were not available. Electron microscopy provided direct evidence that the atypical lymphoid cells were different from the large granular lymphocytes. However, the recent availability of NK marker antibodies for paraffin sections [15] should be of great help to clarify this situation. In our case, the reactive NK-like T-cells were identified retrospectively by an anti-CD57 monoclonal antibody (Leu 7).

Large granular lymphoproliferative disorder has been reported in association with other hematologic malignancies, such as chronic lymphocytic leukemia, B-cell lymphoproliferative disorder, acute lymphoblastic leukemia, hairy cell leukemia, and acute myelogenous leukemia [6–8], but has not been reported in T-cell lymphoma. A factor has been isolated in B-cell chronic lymphocytic leukemia serum, which promotes proliferation of lymphokine-activated killer (LAK) cells [16]. Presumably, T-cell lymphoma may also produce similar lymphokine and cause NK-like T-cell proliferation as seen in our case. No matter what the mechanism is, this case shows that the NK-like T-cell reaction may confuse the diag-

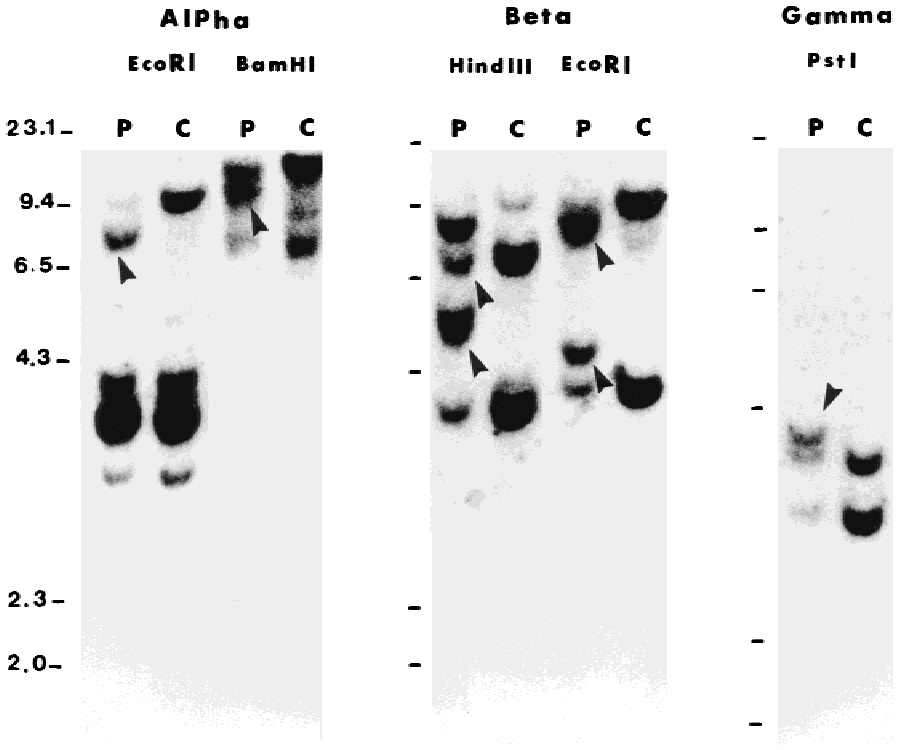

Fig. 4. Southern blot analysis of T-cell receptor (TCR) genes in the peripheral blood specimen. The probes for TCRα,β,γ chain genes identify one to two rearranged bands (arrowheads), after digestion with Eco RI, Bam HI, Hind III or PstI. P = patient specimen; C = control.

nosis and may even lead to a clonal disease, as reported in a case of B-cell lymphoproliferative disorder [8].

Diagnosis of peripheral T-cell lymphoma by flow cytometry frequently depends on the demonstration of selective loss of T-cell antigens, especially the early appearing markers in the maturation process (such as CD7) [17]. However, this phenomenon was not demonstrated in our case. That a high percentage of reactive NK-like T-cells was not demonstrated in the bone marrow was due to the use of a commercial ''NK antibody cocktail,'' which does not include CD57. It appears that a complete set of three major NK markers (CD16, CD56, CD57) should be routinely used when a high percentage of T-cell markers is demonstrated in a lymphoma. It is important to identify the NK component in lymphomas, because an NK-like T-cell lymphoma is usually more aggressive than a peripheral T-cell lymphoma [2,3]. Our case had a rapidly down-hill course, mimicking NK-like T-cell lymphomas, but the cause of death in this patient is apparently due to sepsis rather than the lymphoma per se.

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